CHAPTER 1

INTRODUCTION

1.1 Background of Study

Tempeh is a traditional fermented soybean food. It is a fermented food made by the controlled fermentation of cooked soybeans with a Rhizopus mould as the tempeh starter. The tempeh fermentation by the Rhizopus mould binds the soybeans into compact white cake (Tempeh 2009,25 June). It is made by cooking and dehulling of soybeans and inoculation with different strains of Rhizopus (*R. oligosporus, R. oryzae, and R. stolonifer*) which will lead to solid substrate fermentation (Steinkraus K.H., 1983). Fermentation also influences the content of desirable constituents such as vitamins, protein and fatty acids (Baumann U., 1995).

Tempeh provides the staple food for a large population in Indonesia and Malaysia. Like tofu, tempeh is made from soybeans, but tempeh is a whole soybean product with different nutritional characteristics and also different textural qualities. Tempeh's fermentation process and its retention of the whole bean give it higher content of protein, dietary fiber and vitamins compared to tofu, as well as firmer texture and also with stronger flavor. Because of its nutritional value, tempeh is used worldwide in vegetarian cuisine. Tempeh is a low cost nutritious food and can be consumed by all socio-economic groups.

Figure 1.1 : A piece of uncooked tempe

Table 1.1: Nutritional table for 100g of Tempe

Water	54.9	g
Energy	199	kcal
Energy	833	kJ
Protein	19.0	g
Fat	7.7	g
Sat. fatty acids	1.11	g
Mono-unsat. fatty acids	1.7	g
Poly-unsat. fatty acids	4.3	g
Carbohydrates	17.0	g
Fiber	4.8	g
Ash	1.4	g
Isoflavones	53	mg
Calcium, Ca	93.0	mg
Iron, Fe	2.3	mg
Magnesium, Mg	70.0	mg
Phosphorus, P	206	mg

Potassium, K	367	mg
Sodium, Na	6.0	mg
Zinc, Zn	1.81	mg
Copper, Cu	0.67	mg
Manganese, Mn	1.43	mg
Selenium, Se	8.8	µg
Vitamin C	0.0	mg
Thiamine (B1)	0.131	mg
Riboflavin (B2)	0.111	mg
Niacin (B3)	4.63	mg
Panthotenic acid (B5)	0.355	mg
Vitamin B6	0.299	mg
Folic acid	52.0	µg
Vitamin B12	1.0	µg
Vitamin A	69	µg
[Source: USDA Nutrient Database for Standard Reference]		

From Table 1.1, we can see that tempeh contains a lot of nutrient content that can give a lot of benefits to human body. The most noticeable nutrient content is the amount of protein in the tempeh. With the large amount of protein in tempeh, it makes tempeh popular with the health conscious consumer.

With the numerous and the richness of nutrition in tempeh, it rapidly becomes popular. This is because market nowadays demands for the quality of the food itself which refers back to the nutrient content of the food. Customers nowadays are very well educated and also knowledgeable. However, in Malaysia the production of tempeh is mainly from the small scale industries with traditional way of making. Therefore, the specific facts and also figures of the nutrient content in the traditionally made tempeh are still uncertain due to some reason such as contamination.

1.2 Problem Statement

A lot of products in the market nowadays have their own nutritional labelling attach to their packaging. This gives the customers choices on how to choose their food preferences. However, as in the previous part, major production of tempeh in Malaysia is made traditionally. Therefore, there is no nutritional labelling that is attached to the packaging. This is because; there are no proper scientific data of the nutritional facts on tempeh from the entrepreneurs (small scale industrialist) themselves.

Nowadays tempeh has been gaining lots of attention from either local or overseas researchers on its nutrient content. There are a lot of research planned or been done on tempeh. The main reason for this attention is because of its high nutrient content. There has been research done on the nutrient content of tempeh. However, the research was done under the perfect condition of tempeh and under controlled condition. For this study, the tempeh that will be studied are made from the small scale producer from Kuantan area.

The perfect condition for tempeh is about 36-48 hours after fermentation. That is where the nutrient content of tempeh is at its peak condition. However, in this study also, the nutrient content of aged tempeh also will be studied. Since the utilization of the nutrient content in tempeh is actually from the fermentation process, this study will also check on the effect of fermentation time to the nutrient content in tempeh.

For the determination of nutrient content in tempeh, the method use is by using physical method which is by using Near-Infrared (NIR). However, before the analysis by using NIR can be done, the tempeh samples must first be analyzed by using primary method to set a standard analysis in NIR. However, after setting the standard analysis and analyzing the tempeh samples by s using NIR, there are slight difference on the result between NIR analysis and primary method analysis. In this study, the difference of the analysis will be analyzed.

1.3 Objectives

The objective of this study is to determine the nutrient content of tempeh and also to compare between NIR analysis and primary method analysis. The comparison of nutrient content of tempeh with different packaging such as plastic packaging and paper packaging is also to be studied.

1.4 Scope of Study

To achieve the objectives of this study, these scopes have been identified. The scopes are

i) Focus on the small scale traditional producer
ii) Focus on Kuantan area tempeh producer
iii) Analyzing by using Near-Infrared and primary method
iv) Analyzing between plastic packaging and paper packaging on the nutrient content of tempeh

v) 1 week of fermentation time for analyzing on the effect on the nutrient content

vi) Nutrient element to be studied

a. Protein

b. Fat

c. Fiber

d. Ash

1.5 Rational and Significance

Although the study on the nutritional content of tempeh have been done worldwide, and tempeh has been gaining its popularity among the health conscious customers, in Malaysia, as been told in the previous part major production of tempeh is made traditionally by the small scale industries. Therefore there is not much information that is given to the industrialist about the nutritional facts. They also usually use their own instinct and experience to determine the amount of tempeh starter or vinegar that should be put for the soaking or fermentation process.

Near Infrared (NIR) also has been gaining attention from the industries as the equipment is actually a faster route to do the analysis of nutrient content of samples. However, the analysis using NIR is not yet being approved as one of the reliable analysis to determine the chemical content in the samples. By doing this study, the difference between the primary method analysis and NIR analysis can be studied and analyzed.

By doing this study also, it can helps the small scale industrialist. This is because by using the results from the study, the exact amount of nutrient content in the tempeh produce by these small scale industrialists can actually be determined. From here, the nutritional facts of tempeh produced can be slowly formed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Consumers nowadays usually demand for a wide variety of product which is in high quality, nutritious and also will offer a good value to them. The consumers also very concerned on the safety of the food product that they are buying which we can see nowadays they are testing of food for allergies, pesticides residues and also products from genetic modification of food materials. Many consumers are very interested in the relationship between diet and health. That is why the nutritional facts are very important for every product because these consumers will utilize the nutrient content and health claim information from the food labels to make purchase choices (Kirchner E. M., 1997).

Nutrition labelling regulations differ in countries around the world. In United States, the Food and Drug Administration have made regulations in 1973 where the food must be labelled with regard to their nutritional value. The nutrition label included the following: serving size, number of servings per container, calories per serving, grams of protein, carbohydrate, fat per serving and also percentage of U.S. Recommended Dietary Allowance (USDRA) per serving of protein, vitamins, thiamine, riboflavin, niacin, calcium and iron (Schultz H. W., 1981).

In Malaysia, there are Malaysian Food Act 1983 and Food Regulations 1985, which protect the public against hazard and fraud in preparation, sale and use of food. Currently, the Food Regulations does not require mandatory nutrition labelling for food products, except for special purpose foods such as infant formula and cereal based foods and food that have been enriched or fortified (Kementerian Kesihatan Malaysia 2009, 23 August). Even though in Malaysia there are no specific regulations on the nutritional labelling, the labelling is very important so that the product can create a competitive advantage in the market. Tempeh's product that is produced by the small scale industrialist did not have the nutritional labelling which makes the consumers hesitate whether to buy the product that have the nutritional labelling or not. They are also less competitive because of this lack of information.

2.2 Processing steps of tempeh

From the website on the tempeh production, it stated that there are five processing steps of making tempeh (Tempeh 2009,25 June). The steps are:

1. Cracking the soybeans
2. Soaking and dehulling the soybeans
3. Cooking the soybeans
4. Inoculating the soybeans with tempeh starter
5. Incubating the beans (fermentation process)

2.2.1 Cracking the soybeans

Before the soybeans can be used to make tempeh, the soybeans have to be cracked in half. This will make the process of dehulling easier. To crack the soybeans, usually a loosely set of grain mill is used. There are also stores that sell dehulled soybeans.

2.2.2 Soaking and dehulling soybeans

After cracking the soybeans, the soybeans then soaked in water for 6 to 18 hours or overnight. During the soaking period, the hulls of the soybean will rise at the surface of the soaking water when the soybeans were squeeze by hands with a kneading motion. The water then poured off with the hulls. Then fresh water is added and the step is repeated.

During the soaking process, the first stage of fermentation process also happens. The spontaneous and uncontrolled fermentation of soybeans occurs during the soaking stage where it results because of the fungal fermentation. Usually the fermentation results in an acidification of the beans. The fermentative acidification during soaking inhibits the multiplication of spoilage. However the acidification during the soaking process can be controlled by recycling part of the soak water from the previous batch as inoculums which will result in the soak water pH to be 4.1-4.9 depending on the soaking temperature and recycling rate (M. J. R. Nout, 1987).

2.2.3 Cooking the soybeans

The beans are put in a cooking pot and also water to cover the soybeans. Add the vinegar before cooking the beans and after that the beans were cooked for about 30 minutes. After that the water were drained off and dried the soybeans by continue heating them in the pot on medium heat for a few minutes and until the beans are dry. The soybeans then allowed to cool down to below 35°C.

2.2.4 Inoculating the soybeans with tempeh starter

The soybeans then sprinkled with tempeh starter. The tempeh starter and the beans then mix with a clean utensil to distribute the tempeh starter evenly. To reduce the risk of spoilage and make the fermentation faster, the tempeh starter must be mix very well with the beans.

Figure 2.1: Tempeh starter

Tempeh starter, also called powdered tempeh starter (PTS), is a dried mixture of live Rhizopus spores with substrate, which can be soybeans or rice. Tempeh starter will push the process into the desired direction. In tempeh fermentation, to produce good quality tempeh, tempeh starter with a very high count of desirable Rhizopus molds is needed. Tempeh can be produced by two Rhizopus strains: Rhizopus oryzae or Rhizopus oligosporus, both of which can be isolated from fresh Indonesian tempeh (Tempeh 2009,25 June).

Tempeh starter can be divided into two types which is Indonesian style (traditional) and Western style (modern). In Indonesia, where tempeh originated and is still produced in small tempeh shops, the tempeh master always uses dried tempeh starter. They make it by placing a handful of cooked and inoculated soybeans between two hibiscus leaves, allowing them to incubate for a few days until the soybeans are covered with black spores and finally drying them in the sun. They use this starter by rubbing the hibiscus leaves above the soybeans to be inoculated. As you can understand, this type of tempeh starter can easily be contaminated with other molds or bacteria. However, the climatic conditions in Indonesia are so ideal for tempeh fermentation that this type of contamination is not known to cause problems.

In Western countries, where tempeh production is rather new, tempeh factories always use pure cultures to make sure that the quality of the finished tempeh is consistent and to minimize the risk of failed batches. There are no specific legal standards for tempeh starter, but good quality tempeh starter should contain millions of Rhizopus spores, contain no contaminating, coliform or pathogenic bacteria. Tempeh starter is often extended with sterile rice flower or starch to standardize the spore count.

2.2.4.1 Rhizopus Moulds

Tempeh contains natural antibiotics. This is because the Rhizopus moulds in the tempeh starter produce natural, heat-stable antibiotic agents against some disease-causing organisms. The process of fermentation makes the soybeans softer, since enzymes produced by the mould predigests a large portion of the basic nutrients. The Rhizopus moulds produce an enzyme phytase which breaks down phytates, thereby increasing the absorption of minerals such as zinc, iron and calcium (Tempeh 2009,25 June).

2.2.5 Incubating the beans (fermentation process)

The well mixed soybeans and also tempeh starter then put into plastic bags. The plastic bags have been perforate with holes at a distance of about 1 cm. This is done to make sure that the mould could breathe. The packed beans then placed in an incubator at 30°C or at a warm place for about 36 to 48 hours. During this time, tempeh fermentation will takes place. Then the container should be filled completely with white mycelium and the entire contents can be lifted out as a whole piece.

Processing of soybeans into tempeh (fermentation process) brought about favorable nutritional changes including reduction in the level of phytic acid, starch and the flatulence-causing oligosaccharides stachyose and raffinose; whereas thiamine concentrations were reduced, riboflavin and nicotinic acid contents increased during fermentation (W. B. Van der Piet, 1987).

2.3 Nutrient Content of tempeh

Even though tempeh can be categorized as one of the cheap, basic foodstuff in Indonesia and also in Malaysia, it contains a lot of nutrient that is very good for human health. The fermentation process that is used to make tempeh influences the nutrient content. The increased content of some vitamins of the B-group, especially riboflavin, niacin, vitamin B6 and vitamin B12 is due to the fungal and bacterial activities during the fermentation process. Even though the finished tempeh contains high nutrient content, it stated in the research on the changes of the content of fat during tempeh fermentation that there are some nutrient loses from the finished tempeh due to the preparatory treatment of soybeans before the fermentation process (J. Denter, 1998).

2.3.1 Protein

Protein is one of the main components in soybean. From Table 1.1 in chapter 1, we can see that there are slight differences in the protein content in raw soybeans and also in tempeh. In raw soybeans, there are about 36.49g of protein in it while in tempeh the amount is reduced to 19g. This happens because of the process happens in between before the soybeans become tempeh. Proteins are an abundant component in all cells, and almost all except storage proteins are important for biological functions and cell structure. Food proteins are very complex. They are composed of elements including hydrogen, carbon, nitrogen, oxygen and sulfur. (S. Suzanne Nelson, 2003). The protein in tempeh is excellent for diabetic patients, who tend to have problem with animal sources of protein. The protein and fiber in tempeh can also prevent high blood sugar levels and helps in keeping blood sugar level under control.

Protein serve as the building material of muscles and other animal tissues and in plants, they play crucial metabolic roles as enzymes and enzyme inhibitors, participate in the transport and binding of oxygen and metal ions and perform immunological functions. During their development, cereal grain and legume seeds deposits large quantities of storage proteins in granules known also as protein bodies. In soybeans, these proteins constitute 60-70% of the total protein content and the granules in 80% are made of proteins (Zdzislaw E. Sikorski, 2002).

In a research paper wrote by Sparringa and Owens, the research paper mainly aims on to identify until to what extent the proteins were utilized during the fermentation process of tempeh. The experiment was done by fermenting bacteria free tempeh which is prepared with acidified soybeans cotyledons and *Rhizopus oligosporus* at 30 degree C. From here, the protein oxidation which is estimated from the ammonia production, was 5g at 24hours, 10g at 46 hours. The total amount of soy protein hydrolyzed, including the one that is incorporated into mould biomass, was estimated to be 80g at 28 hours incubation, 95 g at 46 h, and 100 g at 72 h. The hydrolyzed protein at 46 h represented 25% of the initial protein. Of this hydrolyzed protein, it is suggested that approximately 65% remained in the tempeh as amino acids and peptides, 25% was assimilated into mould biomass, and 10% was oxidized (Sparringa R. A., 1999).

2.3.2 Ash

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. Ash content represents the total mineral content in foods. The ash content of most fresh foods rarely is greater than 5%. Pure oils and fats generally contain little or no ash, products such as cured bacon may contain 6% ash, and dried beef may be as high as 11.6%. Fats, Oils and shortenings vary from 0.0 to 4.09% ash, while dairy products vary from 0.5 to 5.1%. It would be expected that grain and grain products with bran would tend to be higher in ash content than such products without bran. Nuts and nut products contain 0.8-3.4% ash, while meat. Poultry and seafood contains 0.7-1.3%. In finished tempeh there is about 1.4g of ash (S. Suzanne Nelson, 2003).

2.3.3 Moisture

In soybeans : Water 8.54 g

In tempeh :Water 54.9 g

(Source : USDA Nutrient Database)

As we can see from the nutrient table of both raw soybeans and also tempeh, we can see that the water content greatly increases from before the fermentation process and after the fermentation process to become tempeh. Because of high water content in tempeh makes it easily to cause spoilage. This is because the microbial growth has always linked to the water activity. This is why when preparing the soybean to be fermented, it is very crucial to make sure that there are no contamination happens to avoid spoilage.

2.3.4 Fat

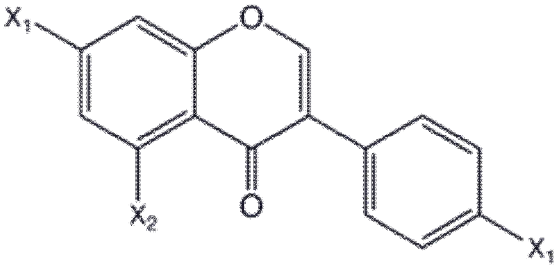
Fats consist of a wide group of compounds that are generally soluble in organic solvents and largely insoluble in water. Chemically, fats are generally triesters of glycerol and fatty acids. Fats may be either solid or liquid at normal room temperature, depending on their structure and composition. Although the words "oils", "fats", and "lipids" are all used to refer to fats, "oils" is usually used to refer to fats that are liquids at normal room temperature, while "fats" is usually used to refer to fats that are solids at normal room temperature. "Lipids" is used to refer to both liquid and solid fats, along with other related substances (Maton Anthea, 1993). Fats generally refer to the lipids that are in solid at room temperature.

Foods contain many types of lipids, but those which tend to be the greatest importance are the triacylglycerols and the phospholipids. Liquid triacylglycerols at room temperature are referred to as oils, such as soybean oil and olive oil and generally are from plant origin. Solid triacylglycerols at room temperature are termed as fats (S. Suzanne Nelson, 2003). Fats impart physical properties to foods and thereby affect the sensory, nutritional, safety and storage characteristics (Richard O. A., 2005).

2.3.5 Fiber

Tempeh contains high fiber content. One serving of tempeh (100g) contains more fiber than most people's consume in one day. Fiber is essential for a healthy digestive tract as well as preventing many chronic diseases.

2.3.6 Isoflavones



Isoflavone (X_1 and X_2 = H),

genistein (X_1 and X_2 = OH) and

daidzein (X_1 = OH, X_2 = H).

Figure 2.2 : Structure of Isoflavones

Isoflavones are phytochemicals, which are compounds found only in plants. They are also a type of phytoestrogen that resembles human estrogen in chemical structure yet are weaker. By mimicking human estrogen at certain sites in the body, isoflavones provide many health benefits that help you to avoid disease. Isoflavones are found in soybeans, chick peas and other legumes. However, soybeans are unique because they have the highest concentration of these powerful compounds.

Table 2.1 : Isoflavones levels in some foods (per 100g):

Tempeh	**43.52 mg**
Peas bean	2.42 mg
Peanuts	0.26 mg
Navy bean	0.20 mg
Chickpeas	0.10 mg
Lentils	0.10 mg
Bread	0.02 mg
Black bean	0.00 mg

(Source:USDA-Iowa State University Database on the Isoflavone, Rel. 1.3 - 2002)

From the table above we can see that tempeh (fermented soybeans) contains the highest isoflavones. Some of the health benefit of isoflavones are they can lower the cancer risk, improved bone health, relieves menopause symptoms and also lowers cholesterol.

Soy contains many isoflavones, but the most beneficial are Daidzein (Da) and Genistein (Ge). When the tempeh is fried, the Da and Ge contents significantly decreased as much as 21% and 58%, respectively, compared to raw tempeh. Heat applied during tempeh frying caused the decarboxylation of the compounds (Hasnah Haron, 2009).

2.3.7 Carbohydrates

Carbohydrates are important in foods as major source of energy, as imparters of crucial textural properties, and as dietary fiber which influences physiological processes. Digestible carbohydrates, which are converted into monosaccharides, which are absorbed, provide metabolic energy. Worldwide, carbohydrates account for more than 70% of the caloric value of the human diet. It is recommended that all person should limit calories from fat(the other significant source) to not more than 30% and that most of the carbohydrate calories should come from starch (S. Suzanne Nelson). Therefore, from the amount of carbohydrates contained in tempeh and also how these carbohydrates came from natural sources and starch, tempeh could and should be one of the choices that people will choose to make a healthy diet.

2.4 Near Infrared (NIR)

Near infrared (NIR) spectroscopy extracts usable information from the absorption spectral characteristic of a sample irradiated by light in the NIR region (G. Dotzlaw, 1993). The NIR region (780 – 2500 nm) is dominated by overtone and combination bands of fundamental vibrations occurring in the mid infrared.

It has been recognized that NIR reflectance is sensitive to particles size, shape and distribution of powders of granular samples (W. W. Wendtlandt, 1966). A sensor that has sensitivity to two measureable quantities is said to exhibit cross-sensitivity. For these sensors, calibration involves maximising the wanted and minimising the unwanted signal. For primary NIR reflectance applications, i.e. determining the chemical compounds in granular or powdered samples, particles size effects on the spectra are considered as the unwanted signal or noise. Therefore, to ensure adequate precision in quantitative chemical analysis, the particles size effects are reduced by grinding the sample finely to a near-uniform size followed by proper sampling (E. W. Ciurcza(1986)k, P. C. Williams(1987)).

The cross sensitivity of NIR can be exploited for particle size analysis. Due to its proven reliability and speed in multi-constituent monitoring and control (A. Robertson, 1989), and the availability of fiber optic probes, NIR reflectance is receiving renewed interest as a potential online sensor for particles size. Because only one sensor is needed to monitor both chemical constituents and particles size (J. L. Ilari, 1988), NIR reflectance has advantages in powder analysis over other methods.

2.4.1 Principle of NIR

In NIR diffuse reflectance spectroscopy, the requirements of classical absorption spectroscopy are not completely satisfied because the sample is non-homogenous and scattering (W. F. McClure, 1994). The theory of NIR reflectance spectroscopy is not fully defined, but empirical results show that Beer-Lambert's law holds, at least in principle (W. F. McClure1994).

An NIR beam incident on a powdery or granular material of a weakly-absorbing medium, thick enough to prevent transmission, will penetrate the layer and its direction will be changed each time a particle boundary is encountered. The changes in direction are a result of reflection, refraction and random diffraction at the surface of various particles. The combination of these effects is called light scattering. As scattered light encounters more boundaries of particles, further scattering occurs in all directions and part of it is absorbed. Scattering and absorption occurs simultaneously in the layer until finally the remaining attenuated light re-emerges from the entry surface. This light is called diffuse reflectance (G. S. Birth, 1987).

Each time the radiation interacts with a sample particle, the chemical constituents in the sample can absorb a portion of the radiation. Therefore the diffusely reflected radiations contain information about the chemical composition of the sample, as indicated by the amount of energy absorbed at specific wavelengths (S. Suzanne Nelson, 2003).

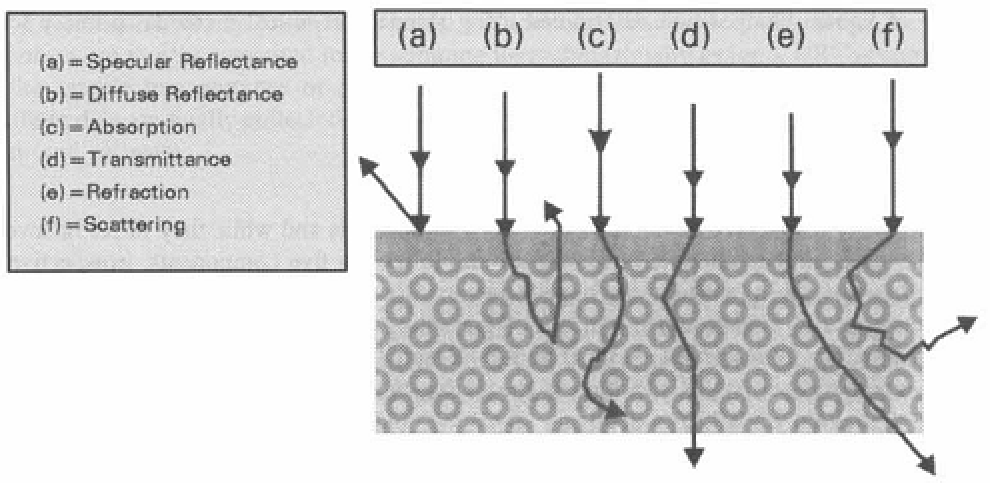


Figure 2.3: Interaction of Near infrared radiation with solid particle in a sample

2.4.2 Application of NIR to Food Analysis

Theory and applications of NIR spectroscopy to food analysis has found its biggest use in the grain, cereal product and oilseed processing industries. NIR techniques using reflectance measurements from ground or powdered samples have been adopted as approved methods of analysis by the American Association of cereal chemist (AACC, 2000.). NIR also can be used for other food products to measure moisture, protein, lactose, monitoring sugar content (S. Suzanne Nelson, 2003).

CHAPTER 3

METHODOLOGY

3.1 Introduction

The overall methodology that is used in this study is:

i) Preparation of tempeh samples.
ii) Standard analysis for tempeh samples.
    a. Protein
    b. Fiber
    c. Fat
    d. Ash
iii) Analysis of tempeh samples using NIR.

The methods used were given by Malaysian Agricultural Research and Development Institute (MARDI)

3.2 Preparation of tempeh samples

In order to do the analysis for the study, the samples are prepared for two purposes:

i) Standard analysis
ii) Analysis using NIR

For the standard analysis by using primary method, the samples were taken from a few kiosks from Kuantan area. These samples then will be duplicate to make sure that the analysis is as accurate as possible. These samples will be analyzed by using the method chosen according to the suitability and availability of materials. A few of the methods are suggested by MARDI. From the results taken from the primary analysis, the results will then be used to do the analysis using NIR where the primary analysis results are used as the standard in the NIR.

For the NIR analysis, the samples were taken from random producers or kiosk from Kuantan area. These samples were taken randomly as for the determination of nutrient content in the tempeh samples that is produced by the small scale producers around Kuantan area.

3.3 Standard analysis for tempeh samples

In this study there are four (4) types of nutrient content that will be studied. The nutrient contents are :

a. Protein
b. Fiber
c. Fat
d. Ash

3.3.1 Standard analysis for Protein

This method is called Protein Determination using Kjeldahl Method. This method can be applied to few type of food samples such as from animal origin, grains and cereals and also legumes.

Reagents:

a. Sulphuric acid (98%)
b. Kjeldahl tablet (5 g each)
c. Sodium Hydroxide
d. pH indicator (0.5 g Bromothymol Blue in 500 mL ethanol (95%) and 500 mL distilled water)
e. Activated charcoal (granular)
f. Boric Acid (4%)

Material and equipment:

a. Kjeldahl digestion and distillation apparatus
b. 500 ml Kjeldahl flasks
c. volumetric flask

Method:

Digestion

Parameter	< 0.5 g sample	1 g sample	< 5 g sample
Sulphuric Acid (98%)	10 mL	20 mL	30 mL
Kjeldahl Tablet (5 g each)	1	2	3
Warm up time	30 min	30 min	30 min
Digestion time	90 min	90 min	90-120 min

Table 3.1: Amount for digestion process

Scrubber

1. To prepare 5 L of sodium hydroxide (8%) (NaOH) : Dissolve 400 g NaOH in 5 L distilled water
2. Add pH indicator
3. Add activated charcoal (granular)

Distillation

1. To prepare 1 L of boric acid (4%) : Dissolve 40 g boric acid in 800 mL distilled water. Adjust to pH 4.65 using NaOH (10 %). Fill up to 1 L with distilled water
2. Tp prepare 1 L sulphuric acid (0.25 M) : Add 13.3 mL sulphuric acid (98%) into volumetric flask and make up to 1 L with distilled water
3. Tp prepare 500 mL NaOH (10 %) : Dissolve 50 g NaOH in 500 mL distilled water

Water	50 mL
NaOH (32%)	90 mL
Reaction time	5 s
Distillation time	240 s
Steam power	100%
Stirrer speed	5^5

Table 3.2: Standard setting on the equipment

Titration

1. Titrate distillate sample from KjelFlex with sulphuric acid (0.25 M) and stop once it reaches slight purple or pH 4.65
2. Record the volume of titrant used

Calculation of % Nitrogen

For 0.25 M sulphuric acid or 0.5 M hydrochloric acid :

$$\% N = \frac{(V_{sample} - V_{blank})\ mL \times 0.05 \times 14.0067}{\text{Weight of sample in g}} \qquad \text{(eq 3.1)}$$